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TETRAGN.002A PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Irena N. Merenkova	Group Art Unit 1643
Appl. No.	:	09/471,703	
Filed	:	December 23, 1999	
For	:	ANALYSIS OF NUCLEOTIDE POLYMORPHISMS AT A SITE	
Examiner	:	Unknown	

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November 13, 2000
(Date)

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PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Prior to examination of the above-referenced application, please amend the application as follows:

IN THE CLAIMS:

Please cancel claims 1-31.

Please add the following new claims:

sub B1 Rule 126

34 ~~32.~~ A method for determining the identity of the polymorphic nucleotide in a target sequence having at least two known variants, comprising:

obtaining a sample comprising said target sequence;

hybridizing a primer upstream of said polymorphic nucleotide;

performing a first extension reaction with said hybridized primer in the absence of a deoxyribonucleoside triphosphate (dNTP) or ribonucleoside triphosphate (rNTP) complementary to said first known variant, but in the presence of at least one dNTP or rNTP

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wherein said at least one dNTP or rNTP includes a dNTP or rNTP complementary to said second known variant and wherein said at least one dNTP or rNTP is not detectably labeled or modified;

performing a second extension reaction with said hybridized primer in the absence of a dNTP or rNTP complementary to said second known variant, but in the presence of at least one dNTP or rNTP, wherein said at least one dNTP or rNTP includes a dNTP or rNTP complementary to said first known variant and wherein said at least one dNTP or rNTP is not detectably labeled or modified; and

analyzing the reaction products of said first extension reaction and said second extension reaction.

~~33~~ 35. The method of claim 32 wherein a plurality of dNTPs or rNTPs is included in said first and second extension reactions.

~~34~~ 36. The method of claim 32 wherein only one dNTP or rNTP is included in said first and second extension reactions.

~~35~~ 37. The method of claim 32 wherein said primer hybridizes such that its 3' end is immediately upstream of the polymorphic base.

~~36~~ 38. The method of claim 35 wherein one dNTP or rNTP is added.

~~37~~ 39. The method of claim 32 wherein the 5' end of said primer comprises a radioactive or non-radioactive tag.

~~38~~ 40. The method of claim 32, wherein said target sequence is amplified *in vitro*.

~~39~~ 41. The method of claim 32, wherein said step of analyzing the reaction products of said first extension reaction and said second extension reaction comprises determining the identity of the incorporated nucleotide which is complementary to said first known variant or said second known variant.

~~40~~ 42. The method of claim 32, wherein said step of analyzing the reaction products of said first extension reaction and said second extension reaction comprises determining the length of said reaction products.

~~41~~ 43. The method of claim 32, wherein said step of analyzing the reaction products of said first extension reaction and said second extension reaction comprises performing a technique selected from the group consisting of chromatography, capillary electrophoresis, microfluidic analysis, and slab gel electrophoresis.

42. The method of claim 32, wherein the reaction products are detected using high performance liquid chromatography.

43. The method of claim 32, wherein the reaction products are detected using capillary electrophoresis.

44. The method of claim 32, wherein the reaction products are detected using an intercalating agent.

45. The method of claim 44, wherein said intercalating agent is ethidium bromide.

46. The method of claim 44, wherein said intercalating agent is an unsymmetrical cyanine dye.

47. The method of claim 32, wherein the reaction products are detected using slab electrophoresis and ultraviolet light.

48. The method of claim 32, wherein the reaction products are detected using slab electrophoresis and a DNA-binding dye.

49. The method of claim 32 wherein said target sequence having at least two known variants comprises a biallelic marker associated with genetic disorders.

50. The method of claim 32, wherein said sample containing a target sequence having at least two known variants is from a diploid organism.

51. The method of claim 32, wherein said first extension reaction is performed with a primer having a first length, and said second reaction is performed with a primer having a second length, said first and second lengths being selected such that said first primer and said second primer and any extension products thereof, can be distinguished from one another.

52. The method of claim 51, wherein the reaction products of said first and second extension reactions are analyzed separately.

53. The method of claim 51, wherein the reaction products of said first and second extension reactions are pooled for analysis.

54. A method for screening a DNA sample for a plurality of target sequences having at least two known variants, comprising:

obtaining a sample comprising a plurality of known target sequences;

hybridizing a primer upstream of each of said target sequences, each primer having a length such that said primer and any extension product thereof can be distinguished from the other primers and any extension products thereof;

performing a plurality of extension reactions wherein each extension reaction contains a single free dNTP or rNTP species complementary to one polymorphic nucleotide of said variant, wherein said single free dNTP or rNTP species is not detectably labeled or modified; and

analyzing the reaction products of each extension reaction.

55. The method of claim 54, wherein said target sequences being analyzed are associated with genetic disorders.

56. The method of claim 54, wherein said sample is from a diploid organism.

57. The method of claim 54, wherein the products of the extension reactions are analyzed separately.

58. The method of claim 54, wherein the products of the extension reactions are pooled for analysis.

59. A kit for use in determining the identity of the polymorphic nucleotide in a target sequence having at least two known variants, comprising:

at least one primer that hybridizes to said target sequence such that its 3' end is upstream of said target sequence;

a reagent for performing a primer extension reaction in the absence of a dNTP or rNTP complementary to said first known polymorphic nucleotide, but in the presence of at least one dNTP or rNTP wherein said at least one dNTP or rNTP includes a dNTP or rNTP complementary to said second known variant and wherein said at least one dNTP or rNTP is not detectably labeled or modified; and

a reagent for performing a primer extension reaction in the absence of a dNTP or rNTP complementary to said second known polymorphic nucleotide, but in the presence of at least one dNTP or rNTP wherein said at least one dNTP or rNTP includes a dNTP or rNTP complementary to said first known variant and wherein said at least one dNTP or rNTP is not detectably labeled or modified.

60. The kit of claim 59, further comprising a detection enhancer for said reaction products.

61. The kit of claim 59, further comprising a purifier for said reaction products.

62. The kit of claim 61, further comprising a detection enhancer for said reaction products.

63. A kit for use in determining the identity of the polymorphic nucleotide in a target sequence having at least two known variants, comprising:

at least one primer that hybridizes to said target sequence such that its 3' end is immediately upstream of said target sequence;

a reagent for performing a primer extension reaction containing a single dNTP or rNTP complementary to said first known polymorphic nucleotide, wherein said single dNTP or rNTP is not detectably labeled or modified; and

a reagent for performing a primer extension reaction containing a single dNTP or rNTP complementary to said second known polymorphic nucleotide, wherein said single dNTP or rNTP is not detectably labeled or modified.

64. The kit of claim 63 further comprising a detection enhancer for said reaction products.

65. The kit of claim 63 further comprising a purifier for said reaction products.

66. The kit of claim 65, further comprising a detection enhancer for said reaction products.

67. A method for determining the identity of the polymorphic nucleotide in a target sequence having at least two known variants, comprising performing a primer extension reaction in the absence of a dNTP or rNTP complementary to one of said polymorphic nucleotides but in the presence of at least one dNTP or rNTP complementary to the other polymorphic nucleotide, wherein said at least one dNTP or rNTP complementary to the other polymorphic nucleotide is not detectably labeled or modified, and detecting the reaction products of said extension reaction.

Remarks

Claims 1-31 have been canceled. New claims 32-67 have been added. Support for these claims may be found in the original claims and throughout the specification. Accordingly, no new matter has been added.

If the Examiner has any questions regarding the above amendments, he is cordially invited to contact the undersigned so that any such questions may be promptly resolved.

Respectfully submitted,

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